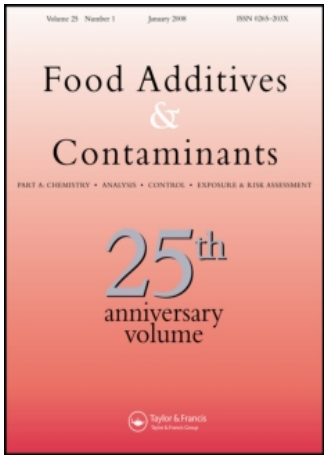

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Wheat kernel black point and fumonisin contamination by *Fusarium proliferatum*

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Abstract
Fusarium proliferatum is a major cause of maize ear rot and fumonisin contamination and also can cause wheat kernel black point disease. The primary objective of this study was to determine whether nine *F. proliferatum* strains from wheat from Nepal can cause black point and fumonisin contamination in wheat kernels. For comparison, the study included three *Fusarium* strains from US maize. In test 1, all the strains but one produced significant symptoms of kernel black point; two strains decreased kernel yield; and four strains contaminated kernels with fumonisins B_1, B_2 and B_3 as determined by liquid chromatography-mass spectroscopy. Strain Ggm202 from Nepal, which produced the highest levels of fumonisins (mean = 49 $\mu g\,g^{-1}$) on five wheat cultivars in test 1, was confirmed to produce fumonisins (mean = 38 $\mu g\,g^{-1}$) on two cultivars in test 2. The data indicate a potential for fumonisin contamination of wheat infected with *F. proliferatum*.

Keywords: *Wheat, durum,* Fusarium proliferatum*, black point disease, fumonisins*

Introduction

Fumonisins are polyketide mycotoxins produced by several *Fusarium* species, especially *F. proliferatum* and *F. verticillioides*, which are common pathogens of maize (*Zea mays* L.) worldwide (Desjardins 2006). The consumption of fumonisins has been shown to cause a number of mycotoxicoses, including leukoencephalomalacia in horses, pulmonary oedema in swine, and liver cancer and neural tube defects in experimental rodents. Consumption of fumonisin-contaminated maize also has been associated epidemiologically with human oesophageal cancer in some areas of the world where maize is a dietary staple. Fumonisins are structurally similar to sphinganine and disrupt sphingolipid metabolism, which is thought to be responsible for the majority of fumonisin-induced mycotoxicoses.

Although a wide range of surveys indicate that maize is the most important source of fumonisin contamination in human food and animal feed, surveys occasionally have reported fumonisins in wheat (*Triticum* species) and wheat-based foods. Shephard et al. (2005) reviewed many of the reports of fumonisins in wheat and concluded that analytical methods and confirmation were insufficient in some of these studies. However, low levels of fumonisins (up to 1.0 $\mu g\,g^{-1}$) in wheat grain and wheat-based foods in Italy have been reported and documented by liquid chromatography-mass spectrometry with selected ion monitoring (Cirillo et al. 2003; Castoria et al. 2005). The fumonisin-producing species *F. proliferatum* has been identified as a major component of the maize ear rot complex and a minor component of the wheat head blight complex in Europe (Bottalico and Perrone 2002; Logrieco et al. 2002). *F. proliferatum* has also been shown to cause black point symptoms on wheat kernels in the USA (Conner et al. 1996). In a survey of *Fusarium* species in cereal grains from smallholder farms in

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Table I. *Fusarium* strains used in the study.

Species	Strain	MAT*	Source	Location
F. proliferatum	Ggm104	NF	Wheat kernel	Lamjung, Nepal
	Ggm118	D-2	Wheat kernel	Lamjung, Nepal
	Ggm140	D-2	Wheat kernel	Lamjung, Nepal
	Ggm171	D-1	Wheat kernel	Lamjung, Nepal
	Ggm181	D-1	Wheat kernel	Lamjung, Nepal
	Ggm184	D-1	Wheat kernel	Lamjung, Nepal
	Ggm185	D-1	Wheat kernel	Lamjung, Nepal
	Ggm187	D-2	Wheat kernel	Lamjung, Nepal
	Ggm202	D-1	Wheat kernel	Kavre, Nepal
	ITEM 2287	D-1	Maize feed	Iowa, USA
	ITEM 2292	D-1	Maize feed	Iowa, USA
F. verticillioides	M-3125	A-1	Maize	California, USA

*D, *Gibberella fujikuroi* mating population D, mating type 1 or 2; NF, not fertile. Fertility data of US strains are from Munkvold et al. (1998) and Proctor et al. (2003).

Nepal, the three dominant *Fusarium* species recovered from wheat, in order of frequency per wheat sample, were *F. graminearum* (56%), *F. equiseti* (52%), and *F. proliferatum* (37%) (Desjardins et al. 2000). In that study, *F. proliferatum* strains were identified by morphology and characterized by sexual fertility as *Gibberella intermedia* (synonym *Gibberella fujikuroi* mating population D), but their plant pathogenicity and mycotoxin production were not investigated.

The primary objective of this exploratory study was to determine whether nine *F. proliferatum* strains from wheat from Nepal can cause wheat kernel black point and fumonisin contamination. For comparative purposes the study included three *Fusarium* strains from US maize: two *F. proliferatum* strains and one *F. verticillioides* strain. Such an analysis should provide information on the potential impact of *F. proliferatum* on wheat quality and safety.

Material and methods

Fungal strains

Eleven *F. proliferatum* strains and one *F. verticillioides* strain (Table I) were used in this study. Strains from Nepal are from a previously described collection (Desjardins et al. 2000). The M-3125 strain of *F. verticillioides* and the two Iowa strains of *F. proliferatum* were selected because a fumonisin gene cluster sequence is available for two of these strains, which will facilitate the eventual goal of functional analysis of fumonisins in wheat black point disease (Proctor et al. 2003; Waalwijk et al. 2004). Strains were routinely cultured on a V-8 vegetable juice medium (Campbell Soup Co., Camden, NJ, USA) and maintained as spore suspensions in 15% glycerol frozen at −80°C.

For fungal isolations from fungal-treated and control spikes of cultivar Fielder from black point test 1, 25 kernels from a pooled sample were surface disinfected by placing them in 0.5% sodium hypochlorite for 1 min and rinsed twice in sterile water. Kernels were placed on a *Fusarium*-selective medium containing 1% pentachloronitrobenzene, and incubated for 5–7 days. Putative *Fusarium* colonies were then subcultured to carnation leaf-agar medium amended with 0.4% potassium chloride and were identified as *F. proliferatum* by formation of microconidia in chains and by other standard morphological criteria (Leslie and Summerell 2006).

Putative *F. proliferatum* strains from Nepal also were identified by determining their *G. fujikuroi* mating population and mating type. Strains were crossed to two tester strains of *F. verticillioides* from Nepal and to standard tester strains of three species obtained from the Fusarium Research Center, The Pennsylvania State University, University Park. Tester strain numbers were: *F. verticillioides* Nep03 (MATA-1) and Nep68 (MATA-2), *F. fujikuroi* M-6884 (MATC-1) and M-6883 (MATC-2), *F. proliferatum* M-6992 (MATD-2) and M-6993 (MATD-1), and *F. thapsinum* M-6564 (MATF-1) and M-6563 (MATF-2). Strains were tested twice as males on carrot agar medium by standard methods (Leslie and Summerell 2006), except that incubation conditions were constant light at 20°C for up to 6 weeks. Crosses were scored as fertile when ascospores were observed upon microscopic examination of the contents of enlarged perithecia.

Identification of *F. proliferatum* from Nepal was confirmed by DNA sequence analysis of the translation elongation factor gene *TEF1*. Genomic DNA was purified from lyophilized mycelia with the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) protocol. Sequencing templates were amplified from genomic DNA by polymerase chain reaction (PCR) and amplification products were purified by the UltraClean (MoBio Laboratories, Solana Beach, CA, USA) method. Sequencing

reactions were done with the BigDye Terminator Cycle (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) protocol. Following the cycle sequencing procedure, reactions were passed through a Sephadex G-50 column, dried under vacuum, suspended in formamide, and subjected to electrophoretic analysis with a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). *TEF1* nucleotide sequence data were submitted to the FUSARIUM-ID database (Geiser et al. 2004). Phylogenetic analysis of *TEF1* sequences was done with the Phylogenetic Analysis Using Parsimony (PAUP) program version 4.0b10 (Sinauer Associates, Sunderland, MA, USA).

Production of fumonisins was assessed in a maize medium consisting of 50 g of cracked maize kernels and 22 ml of water autoclaved in a 300-ml Erlenmeyer flask as previously described (Nelson et al. 1993). Fungal inoculum was produced by washing spores with sterile water from cultures grown on plates of V-8 juice agar medium. Cultures were inoculated with 10^7 fungal spores per flask and incubated in the dark at 28°C for 14 days. Each strain was tested only once in culture because the intent was to survey whether strains were able to produce fumonisins, not to quantitate how much they produced. Fungal inoculum for wheat virulence tests was prepared using 100 ml mung bean liquid medium in 300 ml Erlenmeyer flasks as previously described (Bai and Shaner 1996) and incubated in the dark at 28°C for 4 days on a gyratory shaker at 200 rpm. Spore suspensions (macroconidia and microconidia) were filtered to remove mycelium and were diluted to a concentration of 10^6 spores ml^{-1} in mung bean medium. Control wheat heads were treated with mung bean medium.

Wheat black point assays

Four wheat cultivars were selected based on previous reports of susceptibility to kernel black point (Conner and Thomas 1985; Conner et al. 1996). Seeds of soft white spring wheat (*Triticum aestivum* L.) cultivars Alpowa and Fielder and durum wheat (*T. durum* Desf.) cultivars Plenty and Renville were obtained from the USDA National Small Grains Collection (Aberdeen, ID, USA). Hard red spring wheat (*T. aestivum*) cultivar Wheaton also was included in the tests. Seeds of all cultivars used for planting were free of visible symptoms of black point. All cultivars were grown for 4 weeks in 18-cm plastic pots of a standard pasteurized potting mix in a controlled environment chamber at 15°C with a 12-h light and 12-h dark cycle. The seedlings were thinned to four plants per pot and the pots were moved to greenhouse benches with supplemental lighting. Greenhouse temperatures were set to 23°C during the day and 17°C during the night. After fungal treatment, pots were moved to benches in a plant growth room with the same temperature and lighting regimen. Plants were treated at the early anthesis stage, using ten spikes per treatment and three different treatment methods as previously described (Conner and Thomas 1985; Conner et al. 1996). For spikelet injection, one drop of spore suspension was injected into the central flower of each spikelet on one side of the spike (half-spike method used for test 1) or on both sides of the spike (full-spike method used for test 2). For spray treatment, each spike was sprayed with 3 ml of spore suspension. For dip treatment, each spike was dipped for 5 s in a 50-ml conical tube containing a spore suspension. Five wheat cultivars and 12 fungal strains were used for spike injection treatment in test 1. Two cultivars (Alpowa and Fielder) and three fungal strains were used for spike injection, spray and dip treatments in test 2. After treatment, each head was covered with a plastic bag for 3 days. Spikes were harvested at maturity and were threshed individually by hand. The total kernels in each spike were counted, weighed, and scored for black point disease symptoms as previously described (Conner and Thomas 1985; Conner et al. 1996). Kernels from ten replicate ears of each treatment were pooled and sampled for microbiological and chemical analyses and germination tests. Kernel germination tests were conducted for all treatments in test 1. Fifty seeds from each treatment pool were planted in trays of pasteurized standard potting mix and incubated for 7 days in a plant growth chamber at 22°C with 16-h light and 8-h dark. Seedlings were scored for emergence and for shoot growth of emerged seedlings.

Fumonisin *analysis*

One-half of the pooled kernels from each treatment were ground and extracted for analysis of fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), and fumonisin B_3 (FB_3) by liquid chromatography-mass spectroscopy using a standard method developed for the analysis of maize grain (Plattner 1995).

Results and discussion

Characterization of Fusarium proliferatum

All but one of nine strains of *F. proliferatum* from wheat from Nepal were fertile with tester strains of *F. proliferatum*, but none was fertile with tester strains of *F. fujikuroi*, *F. thapsinum* and *F. verticillioides* (Table I). Both mating types, MATD-1 and MATD-2, were present. Identification of all nine

Table II. Fumonisin levels (combined FB_1, FB_2 and FB_3; $\mu g\,g^{-1}$) in culture and in the kernels of wheat cultivars following treatment with fungal strains.

Fungal strain★	Test†	In culture‡	Cv Fielder¶	Cv Alpowa	Cv Plenty	Cv Renville	Cv Wheaton	Cv mean
Ggm118	1	48	0.03	0.06	0.06	1.25	0.04	0.29
Ggm184	1	850	2.10	2.07	0.14	0.81	0.48	1.12
Ggm104	1	770	0.13	3.24	1.67	0.31	3.05	1.68
Ggm140	1	130	1.23	11.2	0.14	0.03	0.40	2.60
Ggm171	1	280	0.84	4.43	5.24	2.73	0.49	2.75
Ggm181	1	1060	1.87	10.4	3.64	0.37	2.69	3.79
Ggm187	1	710	15.0	4.88	1.41	1.15	1.15	4.72 a
Ggm185	1	690	7.02	9.91	2.02	1.63	3.85	4.89 a
Ggm202	1	730	11.4	21.1	42.9	31.2	140	49 A
ITEM 2287	1	83	5.50	5.16	2.55	2.08	4.38	3.93 a
ITEM 2292	1	3	0.21	5.23	0.62	0.90	1.15	1.62
M-3125	1	3870	0.58	1.77	3.34	1.01	0.05	1.35
Control	1	0	2.24	0.20	0.55	1.09	0.14	0.84
Ggm202	2-1		26.5	101				38 A
	2-2		65.2	8.36				
	2-3		16.9	8.0				
ITEM 2292	2-1		0.11	58.0				10.4
	2-2		2.45	0.11				
	2-3		0.32	1.61				
M-3125	2-1		2.67	3.17				1.14
	2-2		0.33	0.24				
	2-3		0.10	0.37				
Control	2-1		0.55	2.70				0.73
	2-2		0.03	0.39				
	2-3		0.35	0.37				

★Nepal strains are ordered by their fumonisin production *in planta*.
†Tests 1 and 2-1, spike injection; test 2-2, spike spray; and test 2-3, spike dip. ‡Strains were incubated for 2 weeks on cracked maize substrate. ¶Fumonisin analyses were conducted on pools of seeds from ten replicate wheat spikes per treatment. For each test (1 or 2) and strain, means that are significantly different from controls are indicated by an uppercase letter 'A' for $p \leq 0.01$ and a lowercase letter 'a' for $p \leq 0.05$ (Student's paired *t*-test, log-transformation). For statistical analysis of test 2, data for treatment methods 2-1, 2-2, and 2-3 were combined for each strain.

strains as *F. proliferatum* was confirmed by DNA sequence analysis of the *TEF1* gene. All *F. proliferatum* strains from Nepal produced FB_1, FB_2, and FB_3 in culture at levels ranging from 48 to 1060 $\mu g\,g^{-1}$ (Table II). Relative amounts of the three analogues produced by strains from Nepal were similar to previous reports of typical fumonisin production profiles of *Fusarium* species (Rheeder et al. 2002). In cultures of the nine strains, FB_1 was the predominant analogue at 67–88%, followed by FB_2 at 8–28%, and FB_3 at 4–16% of the total amount of fumonisins (FB_1, FB_2, and FB_3) produced. *F. verticillioides* strain M-3125 produced high levels of fumonisins, mainly FB_1, however two US strains of *F. proliferatum* produced only low levels of fumonisins after culture for 2 weeks, in contrast to the high levels that they had previously been reported to produce after culture for 4 weeks (Munkvold et al. 1998).

Wheat kernel black point

The ability of *F. proliferatum* to cause kernel black point was compared by treatment of wheat spikes at anthesis with fungal spore suspensions. Test 1 was a comparison of eleven *F. proliferatum* strains and one *F. verticillioides* strain by injection of spores into spikes of five wheat cultivars. In test 1, all wheat cultivars were susceptible to black point and all but one of the fungal strains produced significant ($p \leq 0.05$) symptoms of kernel black point, as indicated by lower numbers of healthy kernels in fungal-treated spikes than in control spikes (Table III). In all five cultivars tested a high proportion of kernels exhibited mild symptoms of black point; kernels were normal in shape and size but with distinct dark brown or black discoloration of the germ end of the kernel (Figure 1). A smaller proportion of kernels exhibited more severe symptoms, including extension of the brown discoloration into the endosperm, shrunken or shrivelled kernels, and white mycelial growth on the kernel surface. Despite extensive symptoms of black point, only two fungal strains significantly ($p \leq 0.05$) decreased kernel yield and none decreased kernel germination (Table III) or shoot growth of germinated seedlings (data not shown). Among the 12 *Fusarium* strains compared in test 1, *F. proliferatum* strain Ggm202 from wheat from Nepal produced

Table III. Effect of fungal treatment on wheat kernel disease parameters, test 1.

Fungal strain	Healthy kernels (%)*, mean ± SD	Kernel yield (%)†, mean ± SD	Germination (%)‡, mean ± SD
Ggm118	73 ± 32	92 ± 13	80 ± 10
Ggm184	49 ± 23 A	91 ± 16	86 ± 9
Ggm104	48 ± 16 A	79 ± 14	80 ± 10
Ggm140	51 ± 16 A	87 ± 8	68 ± 24
Ggm171	39 ± 21 A	86 ± 20	68 ± 23
Ggm181	36 ± 20 A	79 ± 17	76 ± 21
Ggm187	37 ± 14 A	75 ± 7 A	77 ± 11
Ggm185	42 ± 17 A	91 ± 9	75 ± 20
Ggm202	25 ± 8 A	60 ± 10 A	58 ± 24
ITEM 2287	60 ± 20 a	96 ± 14	86 ± 10
ITEM 2292	58 ± 21 a	90 ± 18	73 ± 32
M-3125	57 ± 17 A	85 ± 15	73 ± 31
Control	96 ± 2	100	86 ± 16

*Mean of five cultivars, spike injection. Based on the number of healthy kernels per spike divided by the total number of kernels in control spikes, ten replicate spikes were injected per treatment. For controls of Alpowa, Fielder, Plenty, Renville, Wheaton, respectively, the number of kernels per spike was 38, 38, 34, 40, and 28. Within each column, means that are significantly different from controls are indicated by an uppercase letter 'A' or a lowercase letter 'a' as in Table II (Student's paired *t*-test).
†Based on total weight of kernels per spike divided by the total weight of kernels in control spikes. For controls of Alpowa, Fielder, Plenty, Renville, Wheaton, respectively, spike yield (g) was 1.43, 1.48, 1.34, 1.49, and 1.19. ‡Based on the number germinated of 50 seeds per treatment. For controls of Alpowa, Fielder, Plenty, Renville, Wheaton, respectively, germination (%) was 62, 90, 90, 22, and 100. Due to low and inconsistent germination, Renville data were not included in the germination means.

Figure 1. Wheat kernel black point symptoms. A, C, and E, controls; B, D, and F, treated with *Fusarium proliferatum* strain Ggm202; A and B, cultivar Wheaton; C and D, cultivar Alpowa; and E and F, cultivar Renville.

the most severe symptoms of black point (Figure 1) with mean reductions of 75% in number of healthy kernels per spike and 40% in weight of kernels per spike (Table III). Test 2 was a further comparison of strain Ggm202 and two US strains by treatment of two cultivars by spike injection, spray, and dip. Cultivars Alpowa and Fielder were selected for test 2 because they had appeared in test 1 to be more susceptible than the other wheat cultivars to fumonisin contamination. All three of the fungal strains produced significant ($p \leq 0.01$) symptoms of kernel black point in test 2, but only strain M-3125 significantly ($p \leq 0.05$) decreased mean

Table IV. Effect of fungal treatment on wheat kernel disease parameters, test 2.

Fungal strain	Healthy kernels (%)*, mean ± SD	Kernel yield (%)†, mean ± SD
Ggm202	25 ± 21 A	66 ± 35
ITEM 2292	34 ± 23 A	72 ± 31
M-3125	26 ± 14 A	67 ± 19 A
Control	90 ± 5	100

*Mean of two cultivars, Alpowa and Fielder, and three inoculation methods, spike injection, spray, and dip. Healthy kernels calculated as in Table III. Control spike kernel numbers were 23 for Alpowa and 35 for Fielder. Statistical analysis is as in Table III.
†Kernel yield calculated as in Table III. Control spike yields were 0.5 g for Alpowa and 1.0 g for Fielder.

kernel yields for the two cultivars and the three treatment methods in test 2 (Table IV).

Fungal-treated plants of cultivar Fielder from test 1 yielded high proportions of kernels infected with *Fusarium* isolates: 64% of kernels from plants treated with *F. proliferatum* strain Ggm202, 52% of kernels from plants treated with *F. proliferatum* strain ITEM 2292, and 92% of kernels from plants treated with *F. verticillioides* strain M-3125. *Fusarium* isolates were recovered both from healthy kernels and from discoloured kernels from treated plants; all isolates were indistinguishable by morphology from *F. proliferatum* and *F. verticillioides*. In tests 1 and 2 control plants yielded occasional kernels with mild symptoms of black-point (Tables III and IV, and Figure 1). Mean percentages of symptomatic kernels from control plants were less than 5% for four of the cultivars tested and 9% for cultivar Fielder. In test 1, 12% of kernels from control plants of cultivar Fielder were infected with *Fusarium* isolates indistinguishable by morphology from *F. proliferatum*. This indicates that while precautions were taken to prevent contamination in the plant growth room, some transfer of inoculum from fungal-treated plants to control plants apparently occurred.

Fumonisin *contamination of wheat*

Among the 12 *Fusarium* strains compared in test 1, strains Ggm185, Ggm187, and Ggm202 from wheat from Nepal and strain ITEM 2287 from maize from Iowa produced significant ($p \leq 0.05$) levels of fumonisins in wheat kernels with black point disease (Table II). Strain Ggm202 produced the highest levels of black point disease and consistently produced fumonisins, with a mean of 49 $\mu g\,g^{-1}$ on the five wheat cultivars in test 1. In wheat kernels, strain Ggm202 produced FB_1, FB_2, and FB_3 at means of 80, 12, and 8%, respectively, of the total amount of fumonisins (combined FB_1, FB_2, and FB_3) produced. Strain Ggm202 also produced high levels of fumonisins in test 2, with a mean of 38 $\mu g\,g^{-1}$ for two cultivars and three treatment methods (Table II). Although *F. proliferatum* and *F. verticillioides* strains from US maize were able to cause wheat black point in tests 1 and 2, only strain ITEM 2287 in test 1 produced a significant level (mean of 3.93 $\mu g\,g^{-1}$) of fumonisins in wheat kernels (Table II). Background levels of fumonisins were relatively low in kernels from control wheat spikes, with means of 0.84 and 0.73 $\mu g\,g^{-1}$ in tests 1 and 2, respectively, but with values as high as 2.4 and 2.7 $\mu g\,g^{-1}$ in two of the 11 control samples. Fumonisins in controls were likely due to cross-contamination of wheat spikes by *F. proliferatum* while black point assays were being conducted in the plant growth room.

As far as we are aware, the present study is the first to demonstrate that genetically defined strains of *F. proliferatum* can cause black point and fumonisin contamination in wheat kernels. Fumonisin contamination levels (combined FB_1, FB_2, and FB_3) of up to 140 $\mu g\,g^{-1}$ were obtained by treating wheat spikes at anthesis with fungal spores. These fumonisin levels are similar to the fumonisin levels (combined FB_1 and FB_2) reported for maize ears experimentally inoculated with *F. proliferatum*, with annual means ranging from 60 to 150 $\mu g\,g^{-1}$ for field tests in Poland in 1996, 1997, and 1999 (Pascale et al. 2002). These preliminary data indicate a significant potential for fumonisin contamination of wheat in which *F. proliferatum* is present. Surveys are underway to determine the natural occurrence of *F. proliferatum* and fumonisins in US wheat with black point disease.

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References

Bai GH, Shaner G. 1996. Variation in *Fusarium graminearum* and cultivar resistance to wheat head scab. Plant Disease 80:975–979.

Bottalico A, Perrone G. 2002. Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. European Journal of Plant Pathology 108:611–624.

Castoria R, Lima G, Ferracane R, Ritieni A. 2005. Occurrence of mycotoxin in farro samples from southern Italy. Journal of Food Protection 68:416–420.

Cirillo T, Ritieni A, Galvano F, Amodio Cocchieri R. 2003. Natural co-occurrence of deoxynivalenol and fumonisins B_1 and B_2 in Italian marketed foodstuffs. Food Additives and Contaminants 20:566–571.

Conner RL, Hwang SF, Stevens RR. 1996. *Fusarium proliferatum*: A new causal agent of black point in wheat. Canadian Journal of Plant Pathology 18:419–423.

Conner RL, Thomas JB. 1985. Genetic variation and screening techniques for resistance to black point in soft white spring wheat. Canadian Journal of Plant Pathology 7:402–407.

Desjardins AE. 2006. *Fusarium* mycotoxins: Chemistry, genetics, and biology. St Paul, MN: American Phytopathological Society Press.

Desjardins AE, Manandhar G, Plattner RD, Maragos CM, Shrestha K, McCormick SP. 2000. Occurrence of *Fusarium* species and mycotoxins in Nepalese maize and wheat and the effect of traditional processing methods on mycotoxin levels. Journal of Food and Agricultural Chemistry 48: 1377–1383.

Geiser DM, delMar Jimenez-Gasco M, Kang S, Makalowska I, Veeraraghavan N, Ward TJ, Zhang N, Kuldau GA, O'Donnell K. 2004. FUSARIUM-ID v. 1.0: a DNA sequence database for identifying *Fusarium*. European Journal of Plant Pathology 110:473–480.

Leslie JF, Summerell BA. 2006. The *Fusarium* laboratory manual. Ames, IA: Blackwell.

Logrieco A, Mulè G, Moretti A, Bottalico A. 2002. Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe. European Journal of Plant Pathology 108: 597–609.

Munkvold G, Stahr HM, Logrieco A, Moretti A, Ritieni A. 1998. Occurrence of fusaproliferin and beauvericin in *Fusarium*-contaminated livestock feed in Iowa. Applied and Environmental Microbiology 64:3923–3926.

Nelson PE, Desjardins AE, Plattner RD. 1993. Fumonisins, mycotoxins produced by *Fusarium* species: Biology, chemistry, and significance. Annual Review of Phytopathology 31:233–252.

Pascale M, Visconti A, Pronczuk M, Wisniewska H, Chelkowski J. 2002. Accumulation of fumonisins, beauvericin and fusaproliferin in maize hybrids inoculated under field conditions with *Fusarium proliferatum*. Mycological Research 106:1026–1030.

Plattner RD. 1995. Detection of fumonisins produced in *Fusarium moniliforme* cultures by HPLC with electrospray MS and evaporative light scattering detectors. Natural Toxins 3:294–298.

Proctor RH, Brown DW, Plattner RD, Desjardins AE. 2003. Co-expression of 15 contiguous genes delineates a fumonisin biosynthetic gene cluster in *Gibberella moniliformis*. Fungal Genetics and Biology 38:237–249.

Rheeder JP, Marasas WFO, Vismer HF. 2002. Production of fumonisin analogues by *Fusarium* species. Applied and Environmental Microbiology 68:2101–2105.

Shephard GS, Van der Westhuizen L, Gatyeni PM, Katerere DR, Marasas WFO. 2005. Do fumonisin mycotoxins occur in wheat? Journal of Agricultural and Food Chemistry 53:9293–9296.

Waalwijk C, Van der Lee T, De Vries I, Hesselink T, Arts J, Kema GHJ. 2004. Synteny in toxigenic *Fusarium* species: the fumonisin gene cluster and the mating region as examples. European Journal of Plant Pathology 110:533–544.